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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Milbrandt et al. : **GROUP ART UNIT**: 1646

SERIAL NO: 09/473,551 : **EXAMINER**: Chernyshev, Olga N.

FILED: December 28, 1999 :

FOR: :

GFR(alpha)1-RET SPECIFIC :
AGONISTS AND METHODS THEREFOR:

Assistant Commissioner for Patents
P.O. Box 2327
Arlington, VA 22202

DECLARATION OF JEFFREY D. MILBRANDT UNDER 37 C.F.R. 1.132

I, JEFFREY D. MILBRANDT, M.D., Ph.D., the below named Declarant, do hereby declare and state as follows:

1. My name is Jeffrey D. Milbrandt, and I reside at 75 Aberdeen Pl, St. Louis, MO 63105.
2. I received a Bachelor of Science degree in Integrated Studies from the Universtiy of Nebraska in 1974, an M.D. from Washington University School of Medicine in 1978, and a Ph.D. in Biochemistry from the University of Virginia in 1983.
3. I am board-eligible in Pathology and am licensed to practice Medicine in the State of Missouri.
4. I am presently a Professor of Pathology and Immunology and Internal Medicine at Washington University School of Medicine in St. Louis, Missouri. I am also actively involved in the Developmental Biology, Molecular Genetics, and Neurosciences Graduate Programs.
5. For over 20 years, my research has focused on the biological function of neurotrophic factors including the GDNF Family of Ligands (GFLs) comprised of neurturin, persephin, artemin, and GDNF, which are closely related proteins that promote survival of multiple neuronal populations, including dopaminergic neurons, which degenerate in Parkinson's disease, and motor neurons, which are affected in Lou Gehrig's disease.

These factors signal through a receptor complex that comprises the Ret tyrosine kinase and a member of a family of GPI-linked co-receptors termed GFRα receptors.

6. I am the author or co-author of more than 126 research articles related to developmental cell biology, many of which pertain to neurotrophic growth factor signaling, 31 of which are directed specifically to the biology of the GDNF-family of related neurotrophic growth factors.

7. I am also an inventor or co-inventor on approximately 75 U.S. and foreign patent grants and applications directed to neurotrophic growth factor signaling.

8. I am a co-inventor on the instant U.S. Patent Application Serial No. 09/473,551, entitled "GFRα1-RET Specific Agonists and Methods Therefor".

9. I have reviewed the Office Action of paper no. 21, mailed on February 22, 2002. In that Action, the Examiner alleges that "the instant specification fails to provide any evidence or sound scientific reasoning that would support a conclusion that the claimed chimeric peptides of SEQ ID NOs: 23 and 26" can "activate GFRα1-RET, but which do not substantially activate GFRα2-RET or GFRα3-RET." (OA 2/22/02 at p. 3). According to the Examiner, because the examples in the specification use a mouse PSPN chimera, rather than the elected human PSPN chimera, the function of the claimed human PSPN chimera has never been tested. (OA 2/22/02 at p. 4).

10. In my opinion, a person of ordinary skill in the art would reasonably conclude that the human PGP-F2ac (SEQ ID NOs: 23 and 26) can be readily substituted for the mouse PGP-F2ac in the same assays described in the specification (Examples 3, 4, and 5), and the human PGP-F2ac would yield the same result or substantially the same result. Accordingly, the specification of the instant application teaches a person of ordinary skill in the art that the human PSPN chimera, like the mouse PSPN chimera, can activate the GFRα1-RET but does not substantially activate GFRα2-RET or GFRα3-RET. My opinion is supported by the following facts:

    a. The specification of the instant application clearly demonstrates that a PGP-F2ac chimera can activate GFRα1-RET but does not substantially activate GFRα2-RET or GFRα3-RET. As shown in Figure 6 of the instant application, a wild-type PSPN cannot activate GFRα1-RET. As demonstrated in Example 3, after

substitution of the F2a and F2c regions of GDNF into PSPN, the PSPN chimera can activate GFRα1-RET.

b. Example 4 shows that the PGP-F2ac cannot substantially activate GFRα2-RET. As explained in the specification, artemin is the only GDNF family member that can activate GFRα3-RET. (specification at p. 36, line 28). Example 4 shows that the Ha region in artemin confers the ability to activate the GFRα3-RET. Without this region, as observed in PAP-F2ac (which behaves virtually identically to PGP-F2ac in activating GFRα1-RET but not GFRα2-RET; please see Figure 6), the chimera cannot substantially activate GFRα3-RET. The claimed PGP-F2ac does not contain an Ha region from artemin and therefore, a skilled artisan would reasonably expect that it cannot substantially activate GFRα3-RET.

c. The active chimera consists of mouse PSPN with substitutions in the respective F2a and F2c regions from GDNF. The amino acid sequence of the mouse PSPN is more than 85% identical to human PSPN and when conservative substitutions (*i.e.* those amino acids that share common chemical attributes and therefore have similar effects in determining the overall shape of the polypeptide) are considered, it is 94% similar to mouse PSPN. Notably, the critical residues of these factors are identical across species, which is consistent with the observations that GFLs from different species have similar functions. For example, the important F2a sequence (AFDDD) and F2c sequence (YHILRKH) from GDNF, which are incorporated into the chimera of the present application and which provide the GFRα1 selectivity, are identical in GDNF derived from human, mouse, rat, zebrafish, and chicken.

d. There has been no reported evidence of any species specificity among the GDNF family members. On the contrary, the evidence supports cross-reactivity among species. In the present application, the PGP-F2ac was built by substituting into mouse PSPN the F2a and F2c regions from rat GDNF. As described above and in the specification, the mouse PSPN-rat GDNF chimera can activate rat GFRα1-RET or human GFRα1-RET without substantially activating GFRα2-RET or GFRα3-RET. Also, the mouse PSPN-mouse NRTN chimera can activate human

GFRα2-RET and the mouse PSPN-human ARTN chimera can activate mouse GFRα3-RET.

e. Further, a wealth of literature supports the interchangeability of these factors, their respective GFRα coreceptors, and the Ret tyrosine kinase from a variety of species ranging from human to rodent to chicken. For example, in the initial description of the isolation and cloning of GDNF (*Lin et al.* Science 260: 1130-1132 (1993)), the authors showed that both rat and human GDNF promoted survival of rat dopaminergic neurons. In subsequent publications, human and rat GDNF were shown to promote neurite outgrowth and survival of chick sympathetic ganglion neurons (*Trupp et al.*, J Cell Bio 130:137-48 (1995)). Furthermore, human GDNF has been demonstrated to provide functional recovery in parkinsonian monkeys (*Gash et al.*, Nature 380:252-255 (1996)). These examples are not restricted to GDNF, as NRTN also displays no species selectivity. Indeed, the original purification of NRTN from Chinese hamster ovary cells was monitored using a bioassay that utilized neurite outgrowth and survival of rat sympathetic neurons isolated from superior cervical ganglia (*Kotzbauer et al.*, Nature 384:467-470 (1996)). These data demonstrate the equivalence of members of this family from various species in a wide variety of assays as well as demonstrating the lack of any species- restricted functions.

f. In addition, the original description of PSPN demonstrated that mouse PSPN was active in promoting survival of rat dopaminergic and motor neurons (*Milbrandt et al.*, Neuron 20:245-253 (1998)). Recently, it has been shown that PSPN-deficient mice suffer more severe damage to the cerebral cortex after ischemia (i.e. they have increased sensitivity to stroke) than wild type mice (*Tomac et al.* Proc Natl Acad Sci USA 99:9521-9526 (2002)). The authors found that treating these PSPN-deficient mice with human PSPN protected them from this increased susceptibility to ischemic damage. The neuronal damage caused by ischemia is the result of excessive glutamate release and subsequent elevation in intracellular calcium levels. The authors went on to show that mouse PSPN and human PSPN function virtually identically, with equivalent dose-response curves, to modulate glutamate-induced calcium influx in cortical neurons. Thus, mouse PSPN and

human PSPN, despite their slight difference in primary sequence homology, have the same effects, both qualitatively and quantitatively, on protection from ischemia and modulation of intracellular [Ca^{2+}] levels.

g. Consistent with the observation of a lack of species specificity in the functions of GFLs, decades of research show that other types of neurotrophic factors, which also exhibit species cross-reactivity, share conserved structural properties. For example, the neurotrophin family of neurotrophic factors (NGF, BDNF, etc.) demonstrates no species specificity in their function and share a highly conserved cysteine knot structure (as do GFLs). The original discovery of NGF resulted from experiments in which a mouse sarcoma was implanted into chicken embryos, whereby the NGF released from the mouse sarcoma produced a massive neural overgrowth of the neurons from the chicken host (*Levi-Montalcini*, R Ann. NY Acad. Sci, 55: 330-343 (1952)). The classical assay used to purify mammalian NGF was the chicken sensory neuron assay. Mouse salivary glands were identified as a preparative source of NGF after it was serendipitously observed that the NGF active on chicken sensory (and subsequently mammalian) neurons was present in snake venom (*Cohen S.*, Proc Natl. Acad Sci USA 46:302-311 (1960)). Like the neurotrophic factors, PSPN and other GFLs are rigidly held molecules due to its conserved cysteine knot structure generated by three internal disulfides within each monomer of the factors. Like the neurotrophic factors, these evolutionarily conserved molecules show virtually no species specificity in their actions.

10. Because members of this neurotrophic factor family, including human PSPN and mouse PSPN, do not show species specificity, and proteins from different species behave similarly in all assays examined thus far, the same GDNF sequences can be inserted into either the human or mouse PSPN to create the appropriate chimeras. A person of ordinary skill in the art would reasonably conclude that a chimera constructed using human PSPN would behave similarly to a mouse PSPN chimera in the same assay. Accordingly, in my opinion, repetition of the examples in the instant application with human PGP-F2ac lacks scientific justification and would be redundant. The lack of species specificity and thus, the species interchangeability of the GFLs are widely

recognized in the field. For example, most investigators in the field using GDNF, NRTN, ARTN, or PSPN (or neurotrophins) do not even identify the species of origin when reporting the results of their experiments. Also, in *Tomac et al.*, Proc Natl Acad Sci USA 99:9521-9526 (2002), the authors performed almost all of their experiments using rhPSPN and did not bother to repeat the same experiments with rmPSPN even though their platform was a mouse model.

11. I further declare that all statements herein made by my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application.

10/15/02
Date

Jeffrey D. Milbrandt, M.D., Ph.D.